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Differential Effects of Pinealectomy on Circadian Rhythms of Feeding and Perch Hopping in the European Starling

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Abstract To study the effects of pinealectomy on the circadian rhythms of locomotor activity and feeding, European starlings (*Sturnus vulgaris*) were held in constant light (0.2 lux and 200 lux) and under constant temperature conditions. Locomotor activity was measured by means of perches with microswitches mounted underneath, and feeding with an infrared photocell system at the feeder. Pinealectomy consistently led to disturbances in perch-hopping rhythms and often to a complete loss of rhythmicity as revealed by periodogram analysis. In some birds, perch-hopping rhythms recovered following a period of initial arrhythmicity. When a perch-hopping rhythm was present, its period was usually shorter than it had been before pinealectomy. In contrast to its effects on perch hopping, pinealectomy had no effect on the persistence of feeding rhythmicity, although its period, like that of the hopping rhythm, decreased after this operation. These results support the hypothesis derived from previous studies that the circadian organization of feeding is different from that of perch hopping. Different circadian pacemakers may be involved, but other models may possibly explain the data just as well.

The pineal organ has been shown to be involved in the control of circadian locomotor activity rhythms in several avian species, particularly passerines. Pinealectomy in house sparrows (*Passer domesticus*; Gaston and Menaker, 1968; Gaston, 1971), Java sparrows (*Padda oryzivora*; Ebihara and Kawamura, 1981), white-crowned sparrows (*Zonotrichia leucophrys*; Gaston, 1971), white-throated sparrows (*Z. albicollis*; McMillan, 1972), and house finches (*Carpodacus mexicanus*; Fuchs, 1983) rendered perch hopping arrhythmic in birds held in constant conditions. In house sparrows, implantation of pineals into the anterior chambers of the eyes of pinealectomized arrhythmic conspecifics restored rhythmicity; the phase of the emerging rhythm was determined by the phase of the rhythm of the donor bird (Zimmerman and Menaker,

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1979). Surgical and chemical denervation of the pineal organ of house sparrows had no profound effects on free-running locomotor activity rhythms (Zimmerman and Menaker, 1975). Pineal organs of house sparrows, chickens, and European starlings (*Sturnus vulgaris*) cultured *in vitro* in continuous darkness (DD) continued to synthesize and secrete melatonin in a circadian fashion for at least two cycles (Binkley *et al.*, 1978; Deguchi, 1979; Kasal *et al.*, 1979; Takahashi *et al.*, 1980; Takahashi, 1981). Taken together, these observations gave rise to the hypothesis that the pineal gland may be the site of a circadian pacemaker that controls locomotor activity via its periodic production and secretion of melatonin. It has been suggested that this melatonin rhythm acts on other damped or self-sustained circadian oscillators lower in a hierarchical system of circadian oscillators (Menaker and Zimmerman, 1976; Gwinner, 1978).

In other avian species, the pineal seems to be much less intimately involved in the control of circadian activity rhythms. In European starlings, removal of the pineal did not necessarily lead to arrhythmicity in DD and dim continuous illumination (LL), although the rhythm apparently became less stable (Gwinner, 1978). Similar results were obtained in pigeons (*Columba livia*; Ebihara *et al.*, 1984). Circadian activity rhythms of chickens (MacBride, 1973) and Japanese quail (*Coturnix coturnix japonica*; Simpson and Follett, 1981; Underwood and Siopes, 1984) were not affected at all by pinealectomy. These results suggest that pacemakers outside the pineal are of primary significance in the control of circadian activity rhythms of these species.

Recent experiments have shown that both the eyes and the suprachiasmatic nuclei (SCN) of the hypothalamus may contain additional pacemakers. In Japanese quail held in DD, enucleation rendered locomotor activity arrhythmic in many individuals (Underwood and Siopes, 1984). The same was true for pinealectomized pigeons (Ebihara *et al.*, 1984). As with pineal glands, retinae of chickens (Binkley *et al.*, 1980; Hamm and Menaker, 1980; Reppert and Sagar, 1983), quail (Underwood *et al.*, 1984), and house sparrows (Binkley *et al.*, 1979) showed rhythms of melatonin and *N*-acetyltransferase (NAT) activity. At least in quail, retinal melatonin was released into the bloodstream, as a plasma melatonin rhythm persisted in pinealectomized birds, but was abolished by blinding (Underwood *et al.*, 1984). The evidence for the SCN containing circadian pacemakers comes from experiments showing that lesions of the SCN abolished circadian activity rhythms of house sparrows (Takahashi and Menaker, 1982), Java sparrows (Ebihara and Kawamura, 1981) and Japanese quail (Simpson and Follett, 1981). These results are consistent with similar findings in mammals, in which there is additional evidence from both *in vitro* and *in vivo* studies demonstrating that the SCN contains a circadian pacemaker (see Moore, 1982, for a review).

Taken together, these data suggest that at least three pacemaker structures, localized in the pineal gland, the eyes, and the SCN, can participate in the control of avian activity rhythms. They may be interconnected in the form of a neuroendocrine feedback loop (Cassone and Menaker, 1984), in which the different components may be of differential significance in different species.

With only one exception (Binkley *et al.*, 1971), all the conclusions drawn about the involvement of the pineal and other pacemakers in the control of avian circadian rhythms have been based on investigations of locomotor activity rhythms. The ques-

tion of whether other circadian rhythms would be affected the same way by experimental manipulations has barely been addressed. Differential responses of different circadian rhythms to experimental treatments seem quite possible. This is especially true in light of recent findings on European starlings indicating that in this species the circadian rhythm of feeding has rather different properties from those of the rhythm of locomotor activity. The rhythm of locomotor activity disappeared in LL of about 10 lux or more, whereas the circadian rhythm of feeding continued up to intensities of at least 1,000 lux. In one bird, perch hopping showed spontaneous splitting, whereas feeding remained in an unsplit state (Gänshirt *et al.*, 1984). Moreover, testosterone implantations increased the duration of the daily perch-hopping activity and sometimes induced splitting of perch-hopping rhythms. The feeding rhythms, however, remained unaffected by this treatment (Subbaraj and Gwinner, 1985). These results suggest that in the starling the circadian organization of feeding and perch hopping may be different, and that perhaps even different circadian pacemakers may be involved. In this paper, we report on the effects of pinealectomy on these two rhythms in starlings exposed to constant conditions.

METHODS

The male European starlings used were captured in southern Germany and held in large outdoor aviaries for at least 1 month prior to the start of the experiments. During the experiments, the birds were held in wire mesh cages (49 × 29 × 36 cm) inside temperature-regulated chambers. Temperature varied between 21°C and 24°C. Food (chick starter mash) and vitamin-enriched water were available *ad libitum*.

Perch hopping activity was measured using microswitches mounted underneath one of the two perches. Feeding was continuously monitored using a photocell system. The infrared light beam was interrupted each time the bird stuck its head into the feeder. The number of occurrences of each activity (hopping and feeding) was recorded separately for every 2-min interval on an electronic data-logging system and later processed by a computer. Standard charts of the daily distribution of hopping and feeding activities were plotted for each bird.

In addition, chi-square periodograms (Sokolove and Bushell, 1978) for successive 2-week intervals[1] were made. In these periodograms, the rhythmicity index Q_p is plotted for periods between 18 and 30 hr, and a line designates significance at the level of $p = 0.001$. On the basis of these periodograms, the percentage of birds that exhibited a "strong" or a "weak" rhythm in hopping and feeding during the successive intervals was determined. A rhythm was defined as "strong" if there was a clear-cut peak of Q_p values that exceeded the significance threshold (as in Fig. 3, below; upper two diagrams). A rhythm was defined as "weak" if only a few Q_p values exceeded the significance level and there was a clear indication of a steady increase of Q_p values followed by a decline (as in Fig. 3, below; lower right diagram). Absence of rhythmicity was assumed either if none of the Q_p values were above the significance threshold or

1. Occasionally shorter intervals were used when there was a recording failure or if there were sudden changes in period violating an important precondition for periodogram analysis.

if only one or a few Q_p values were above the significance threshold, but not associated with a continuous increase and subsequent decline of adjacent Q_p values (as in Fig. 3, below; lower left diagram). For each interval in which a (strong or weak) rhythm could be detected, an estimate of the circadian period (τ) was made. τ was defined as the τ value halfway between the shortest and longest τ value above the significance level.

Pinealectomies and sham operations were performed as described previously (Gwinner, 1978). Histological examinations confirmed that pinealectomies were complete in all the birds used in this study. Two separate experiments were conducted.

EXPERIMENT 1

Seven male starlings were held in individual recording cages in a large (450 × 170 × 270 cm) room. These birds had previously been used for more than 1 year in another experiment in which they had been exposed to LL conditions of either 0.2, 200, 350, or 500 lux, for varying time intervals. On September 27, 1984, the birds were transferred from bright LL (500 lux) to dim LL (0.2 lux), as measured from the bottom of the cage toward the incandescent light source using a light meter. Between November 7 and 11, all birds were pinealectomized. Approximately 3 months after pinealectomy, on February 1, 1985, light intensity was increased to about 200 lux. All birds in this experiment produced useful feeding records. Two birds, however, did not use the recording perches during phases of locomotor activity. Therefore only data on the distribution of feeding are available for these two birds.

EXPERIMENT 2

Nine male starlings were placed in the experimental conditions in April 1985. They were initially held in a light–dark cycle (LD 14:10). After 2–3 weeks, between April 11 and 16, all birds were castrated. This operation was performed to eliminate possible effects of gonadal hormones on the circadian system (Gwinner, 1975a; Gwinner and Turek, 1971; see also "Discussion"). On May 11 they were transferred to dim LL of 0.2 lux. Between July 22 and 24, six birds were pinealectomized and three birds were sham-pinealectomized.[2] On October 15 the light intensity was increased to about 200 lux. This sequence of treatments was exactly parallel to that in Experiment 1. Three pinealectomized and three sham-operated birds were housed in their recording cages in the same (450 × 150 × 220 cm) room; the other three pinealectomized birds were housed individually in soundproof boxes.

RESULTS

Representative recordings of perch hopping and feeding of two starlings (one from each experiment) are shown in Figures 1 and 2. In both birds, initial perch-hopping

2. Results from previous experiments have demonstrated that sham pinealectomy has only minor, if any, effects on the perch-hopping rhythm in starlings (Gwinner, 1978). Therefore only three sham-pinealectomized birds were used as the controls.

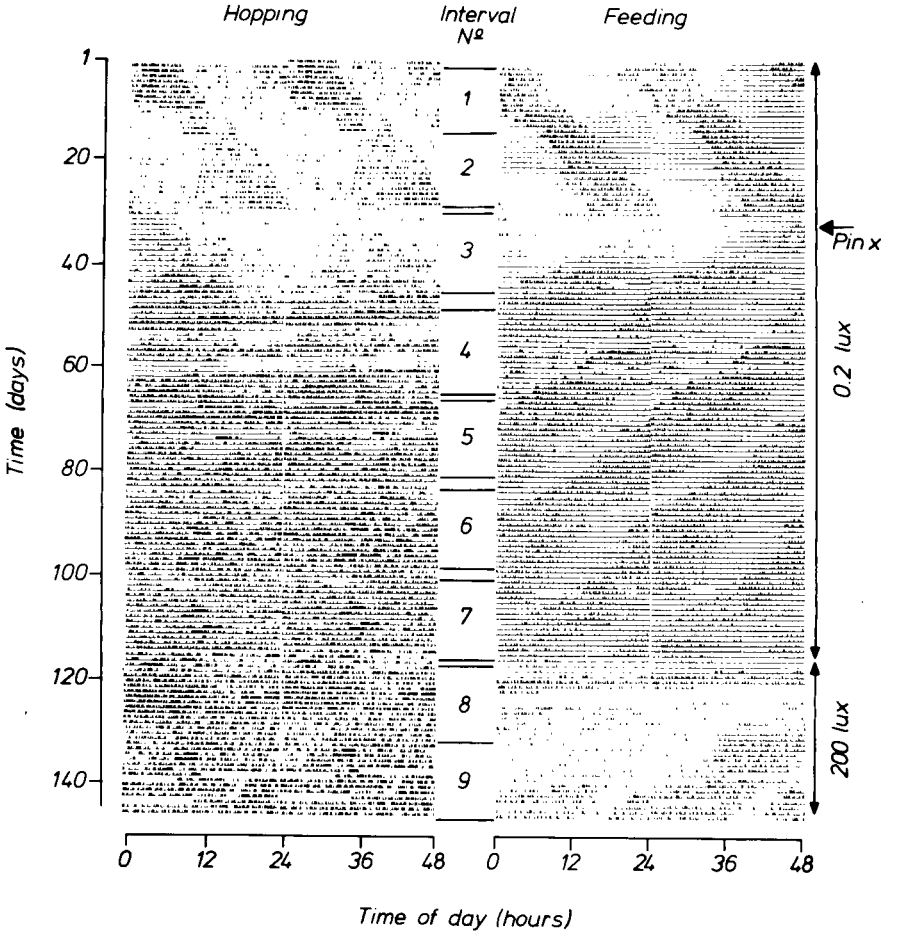

FIGURE 1. Circadian rhythm of perch hopping (left) and feeding (right) of a European starling from Experiment 1. The starling was exposed to dim LL (0.2 lux) for the first 115 days of this record and then to bright LL (200 lux). The bird was pinealectomized on day 30, as shown by the arrow. Each horizontal line from 0 to 24 hr and from 24 to 48 hr represents the activity recording of 1 day. Records from successive 48-hr intervals are mounted below each other in chronological sequence. (Records have been double-plotted on a 48-hr time scale to facilitate inspection of the data.) Periodogram analyses were performed on the nine intervals indicated by the numbers between the two plots.

and feeding activities were rhythmic, but pinealectomy induced changes. Perch hopping became continuous and apparently arrhythmic. In the first starling, shown in Figure 1, arrhythmicity persisted to the end of the experiment. In the second starling, shown in Figure 2, rhythmicity gradually re-emerged starting about 5 weeks after pinealectomy. The τ of the re-emerging hopping rhythm was considerably shorter than before pinealectomy. In both birds, feeding remained rhythmic throughout the experiment; however, following pinealectomy, τ was considerably shorter than before.

In Figure 3, selected periodograms are shown for the records of hopping and feeding of the bird in Figure 1. They confirm the conclusions drawn from inspection

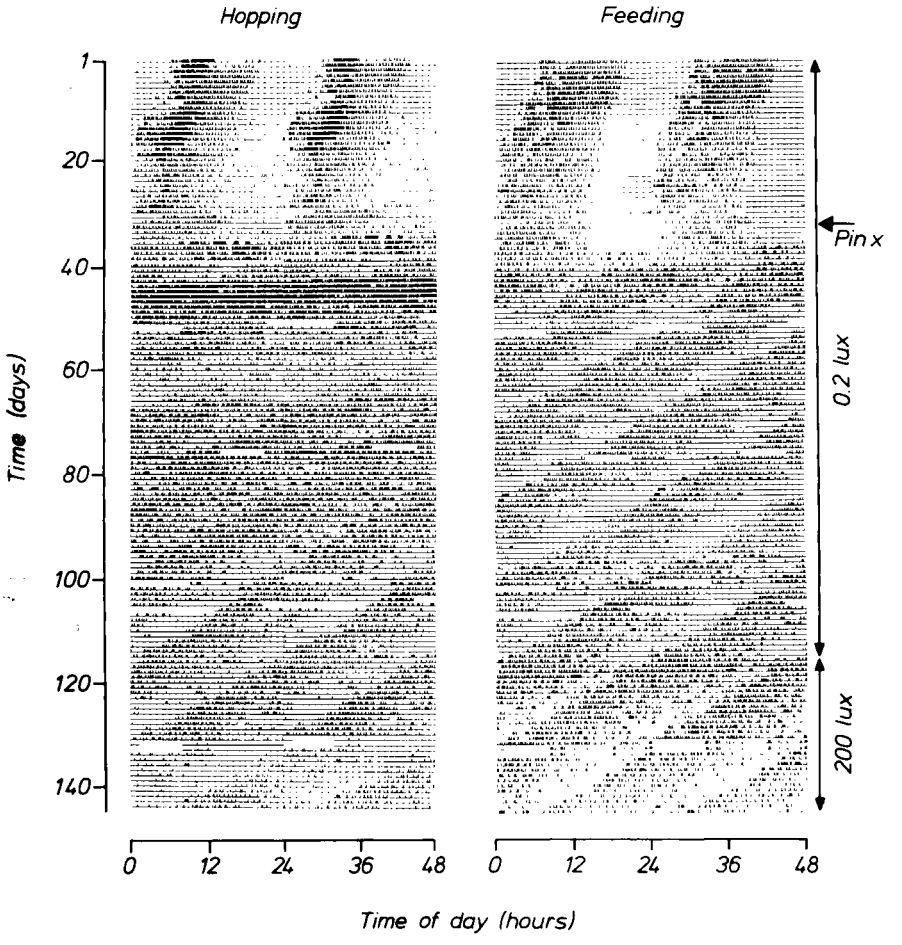

FIGURE 2. Circadian rhythms of perch hopping (left) and feeding (right) of a European starling from Experiment 2. For further explanation, see Figure 1.

of the original activity recording. There were clear peaks in the periodograms for feeding during all intervals shown. In contrast, peaks in the periodograms for hopping were present only before and (with reduced amplitude) immediately after pinealectomy. Subsequently, significant periods were no longer detectable.

Periodograms for both hopping and feeding were calculated for the nine successive 2-week intervals (nos. 1 to 9 in Fig. 1). Then, for all intervals, the percentage of birds was determined that showed rhythmicity (strong or weak; see "Methods"). Results are summarized in Figures 4 and 5. Clearly, feeding was rhythmic in all (Experiment 1) or almost all (Experiment 2) birds throughout the expirement.[3] Perch hopping, in contrast, became arrhythmic in many birds within a few weeks after pinealectomy.

Of those birds that consistently showed strong hopping rhythms in the first two

3. This was also true for the additional two birds of Experiment 1 for which only feeding was recorded. The data of these birds are not included in Figure 3 on the presence of rhythmicity and τ (see below).

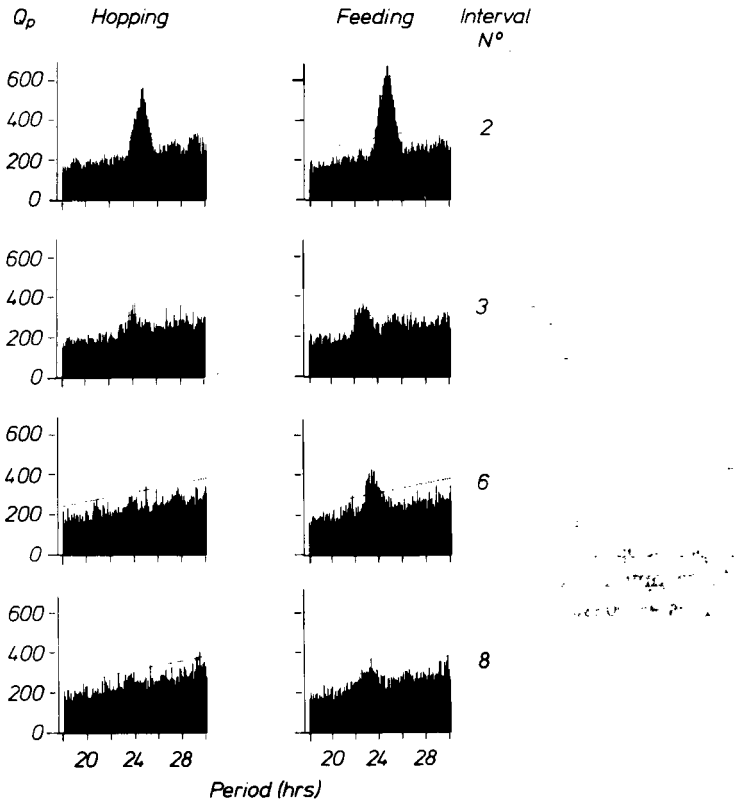

FIGURE 3. Chi-square periodograms of perch hopping (left) and feeding (right) for data from four different 2-week intervals of the recording shown in Figure 1. Q_p values for periods from 18 to 30 hr are plotted. All values above the sloping line are significant ($p < 0.001$) for periodicity. The interval numbers at the right-hand margin correspond to those shown on Figure 1.

time intervals, only one of five birds in each experiment retained strong hopping rhythm in the two time intervals immediately following pinealectomy ($p = 0.05$, sign test). Subsequently, rhythmicity in perch hopping tended to recover in some birds, especially in Experiment 2. Generally, about 50% of all birds remained arrhythmic until the end of the experiment. In the three control birds, in contrast, both feeding and hopping were rhythmic throughout exposure to dim LL (intervals 1–7), and there was only one bird in which perch-hopping rhythmicity was absent during the 2-week period following transfer to bright light (interval 8, Fig. 6).

Changes in the mean τ of the pinealectomized birds are shown in the right-hand diagram of Figures 4 and 5. It is clear that in both experiments there was a tendency for τ to become shorter after pinealectomy. Six of seven birds of Experiment 1 ($p = 0.02$, sign test) and four of five birds of Experiment 2 (n.s.) had shorter circadian periods during interval 3 than during interval 2. This tendency did not occur in the three sham-operated birds (Fig. 6, right-hand diagram); τ increased slightly after sham pinealectomy in two birds and decreased in one bird.

In both the pinealectomized and sham-operated birds, τ tended to shorten after

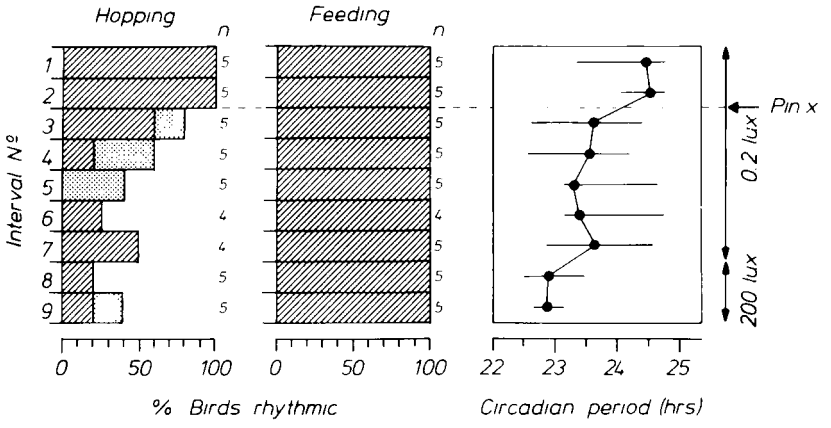

FIGURE 4. Summary diagram of results obtained from those five pinealectomized birds of Experiment 1 from which both perch-hopping and feeding data could be collected. Left and middle diagrams: Percentage of birds that exhibited a "strong" (hatched) or a "weak" (stippled) rhythm in perch hopping (left) and feeding (middle) during the nine successive intervals subjected to periodogram analysis (compare to Fig. 1). Right diagram: Median τ values (with ranges) of the feeding rhythm calculated for the same intervals. The number of starlings on which calculations are based is designated by n.

the transition from dim to bright LL. Actual numbers of pinealectomized and sham-operated birds that had shortened feeding periods after the transition from dim to bright LL are as follows: Experiment 1, six of seven pinealectomized birds (p = 0.02); Experiment 2, three of five pinealectomized birds and two of three sham-operated birds (n.s.). Finally, those birds of Experiment 2 that regained a hopping rhythm also shortened their τ coincident with the transition from dim to bright LL.

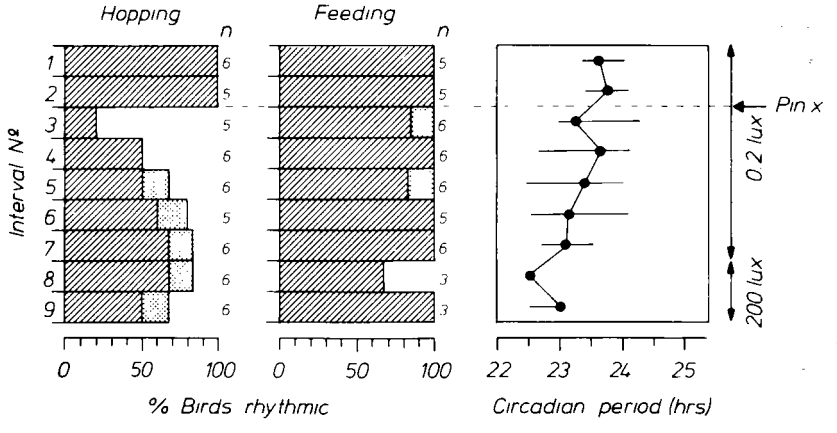

FIGURE 5. Summary diagram of results obtained from the six pinealectomized birds of Experiment 2. For further explanation, see Figure 4.

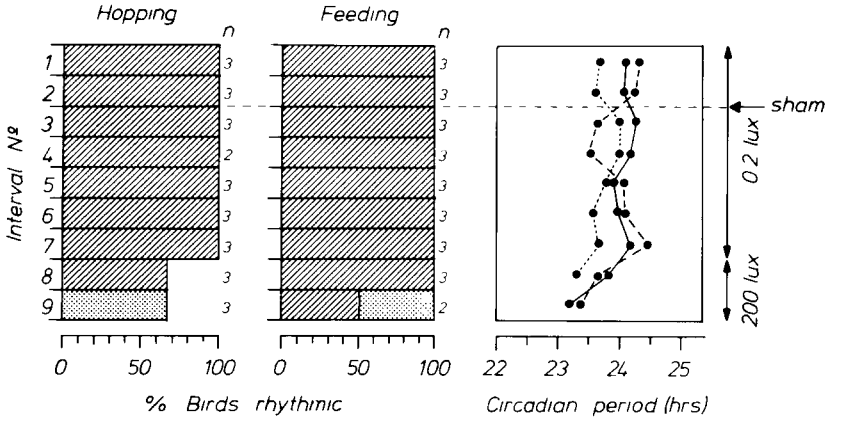

FIGURE 6. Summary diagram of results obtained from the three sham-pinealectomized birds of Experiment 2. On the right diagram, values for individual birds are plotted.

DISCUSSION

The results of the effects of pinealectomy on the circadian rhythms of perch hopping in European starlings are consistent with previous data on the same species (Gwinner, 1978). Sham pinealectomy affected neither the strength nor the period of the rhythm. Pinealectomy, in contrast, consistently led to disturbances in perch-hopping rhythms. In some cases, the disturbances were expressed only in a less pronounced separation between activity times and rest times. In others, they resulted in the complete loss of any detectable rhythmicity. In addition, pinealectomy led to a shortening of τ in most birds.

There were slight differences between the two experiments with regard to the effects of pinealectomy on perch hopping. Particularly, the percentage of birds that became arrhythmic immediately after pinealectomy tended to be higher in Experiment 2 than in Experiment 1. Subsequently, however, rhythmicity recovered in more birds of Experiment 2 than of Experiment 1. It is difficult at present to interpret these differences, but one possible explanation may be related to the fact that the birds of Experiment 2 were castrated, whereas those of Experiment 1 were not. The state of the reproductive cycle of the latter birds is not known precisely, so it is possible that most or all of these birds had active, testosterone-secreting testes at the time of the experiment. This is quite likely, since the bill color of these starlings was monitored and found to be at least partly yellow, which has been shown to be related to elevated levels of testosterone (Witschi and Miller, 1938). Testosterone is known to increase the probability that locomotor activity of starlings will become arrhythmic in constant conditions (Gwinner and Turek, 1971; Gwinner, 1975a).

Another possible explanation for the differences in the perch-hopping behavior of the birds of Experiments 1 and 2 may be found in the fact that the birds of Experiment 1, in contrast to those of Experiment 2, had been living in experimental conditions for more than 1 year prior to the beginning of the experiment. This may

have had effects on their circadian system. In fact, the circadian periods measured at the beginning of the experiments tended to be longer in the birds of Experiment 1 than in those of Experiment 2. This is consistent with other results from starlings indicating a lengthening of circadian period with increasing time in constant conditions (Gwinner, 1975a). It opens the possibility that other properties of the circadian system may also have changed during the extended pre-experimental treatment.

Consistent with Aschoff's rule (e.g., Aschoff, 1979) and with other previous results on the starling (Hoffmann, 1960; Gwinner, 1975b), there was a tendency for τ to decrease when light intensity was increased. This suggests that the processes that lead to the light-induced changes in τ are not dependent on the presence of the pineal. However, more data are required to substantiate this tentative conclusion. The increase in light intensity also led to the slight increase in the percentage of birds (both pinealectomized and sham-operated) showing arrhythmic perch hopping; however, a relatively high proportion of those birds that were rhythmic in dim light remained rhythmic in bright light. Perch-hopping rhythms of starlings tended to disappear under lower light intensities in other experiments (Gwinner, 1975a; Gänshirt *et al.*, 1984). The reason for these differences between experiments is not clear, but they may have been due to differences in the birds' internal physiological state, which is known to have effects on the threshold intensity of perch-hopping arrhythmicity (Gwinner, 1975a,b).

As in a previous experiment with European starlings (Gwinner, 1978), several birds of the present study, particularly those in Experiment 2 (Fig. 4), showed arrhythmic perch hopping during the first weeks following pinealectomy; however, a circadian rhythmicity gradually re-emerged later (Fig. 2). This kind of rhythm recovery may turn out to be an important phenomenon for the understanding of the effects of pinealectomy. Presently, it can be interpreted in several different ways. It may, for instance, reflect the reorganization of a population of slave oscillators that, following abolition of their pacemakers, first become desynchronized from each other but subsequently resynchronize to each other. It may also indicate that other pacemakers take over the role of the pineal after its ablation.

The period of the feeding rhythm was shortened by pinealectomy in the same way as that of the perch-hopping rhythm (when the latter was present). However, in contrast to the perch-hopping rhythm, the feeding rhythm persisted consistently after pinealectomy. Indeed, there was only one bird whose feeding activity was apparently arrhythmic, and this was during only one of the 2-week intervals. Hence, whatever changes pinealectomy induces in starlings' circadian system, the feeding rhythm appears to be less severely affected by these changes. In fact, our results force us to conclude that a pineal pacemaker is not essential for the persistence of the feeding rhythm and that other pacemakers, possibly those in the eyes or the SCN, are significantly involved in its generation.

The differential responses of the feeding and hopping rhythms to other treatments—for example, light or testosterone (Gänshirt *et al.*, 1984; Subbaraj and Gwinner, 1985)—are also consistent with the idea of separate control systems for these two rhythms in the European starling. The available data might be accommodated by a two-pacemaker model for hopping and feeding, but other models are also possible.

For instance, both rhythms might be controlled by one and the same extrapineal pacemaker, but by different slave oscillators that can be differentially affected by pinealectomy, light, and testosterone. More complex models, (e.g., ones based on a population of several interconnected pacemakers; Cassone and Menaker, 1984) are also possible.

In addition, the present data are of interest with regard to a hypothesis first raised by Gaston and Menaker (1968) and subsequently advocated particularly by Rusak (1982). According to this hypothesis, pinealectomy mimics the effects of high light intensity by "modifying the baseline activity level of central targets of photic input and altering photic sensitivity" (Rusak, 1982, p. 47), in the same way as if light intensity were increased. Several observations, including the decrease in τ, the increase in circadian activity time (Gwinner, 1978), and the frequent occurrence of arrhythmicity following pinealectomy are consistent with this idea, as the same changes occur in intact starlings if light intensity is increased (Gwinner and Turek, 1971). Applied to the present results, this model would imply that feeding activity is less sensitive to these effects, either because it is coupled to the pacemaker(s) in a different way than perch hopping or because the processes connecting pacemaker function with overt feeding behavior are less susceptible to the "masking" effects of light and its correlates than those connecting the pacemaker(s) with perch hopping.

Further experiments including studies on the effects of other putative pacemakers (eyes, SCN) are required to clarify the situation. So far, almost all experiments on the effects of the pineal organ and its hormones on avian circadian systems have concentrated on the analysis of locomotor activity rhythms. The only exception is the study by Binkley *et al.* (1971), who showed that pinealectomy in the house sparrow abolished not only the circadian rhythm of hopping but also that of body temperature. In view of the diversity of the effects of pinealectomy on avian circadian systems, it seems promising for future studies to extend the analysis of circadian mechanisms to more than one circadian function.

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